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gene coding for a fluorescent protein wherein the gene coding for the fluorescent protein is expressed in multipotent stem and progenitor cells of the non-human transgenic adult mammal.

REMARKS

Claims 1-3, 9-11, 19 and 22-24 have been amended to overcome the Examiner's objection and to more particularly point out and distinctly claim the invention. New Claims 51-79 have been added to claim specific embodiments of the invention. Support for the amendments can be found throughout the specification and in the originally filed claims. In particular, support for the amendments can be found at page 5, line 22, page 12, lines 16-29, page 10, lines 25 to page 11, line 11 and in examples. No new matter has been introduced.

The remainder of this Reply is set forth under appropriate subheadings, for the convenience of the Examiner.

Restriction Requirement

Applicants acknowledge that Groups II, III and IV have been rejoined with Group I and that Claims 1-24 are under consideration. Applicants respectfully request examination of newly presented Claims 51-79, since these claims are directed to specific embodiments of the invention claimed in Groups II, III and IV. Applicants also acknowledge that Claims 25-50 are withdrawn from consideration and that the restriction requirement has been made final.

Applicants have timely traversed the requirement in the Preliminary Amendment and Reply to Restriction Requirement mailed on September 5, 2000 (Paper No. 12). Applicants reserve the right to file one or more divisional applications or take such action as deemed necessary to protect the inventions classified by the Examiner in Groups V-VII. Applicants do not hereby abandon or wave any rights in the inventions of the non-elected claims.

Double Patenting

The Examiner states that "should claims 1, 6, 7 and 8 be found allowable, claims 17, 22, 23 and 24 will be objected to under 37 C.F.R. § 1.75 as being a substantial duplicates thereof". Specifically, the Examiner states "that Claim 17 is a product by process claim in which the

product is identical to that of claim 1" and that "the mammals of claims 22, 23 and 24 are identical to the mammals of claims 6, 7 and 8, respectively". See Office Action at page 3.

Applicants respectfully submit that the scope of protection afforded by Claim 17 is different from that afforded by Claim 1. Specifically, Claim 17 is directed to a non-human transgenic mammal produced by the method of Claim 9, whereas Claim 1 is not limited to any particular method for producing the non-human transgenic mammal. Therefore, Claims 1 and 17 differ substantially from each other and are not unduly multiplied. 37 C.F.R. § 1.75.

Applicants have amended Claims 22, 23 and 24 to recite "the method of Claim 19" rather than "[t]he non-human transgenic mammal, progeny or embryo thereof of Claim 19". Applicants respectfully submit that, as amended, Claims 22, 23 and 24 are not substantial duplicates of Claims 6, 7, and 8, respectively.

Rejection of Claims 1-17 and 19-24 Under 35 U.S.C. § 112, First Paragraph

Claims 1-17 and 19-24 are rejected under 35 U.S.C. § 112, first paragraph. The Examiner states that the specification "does not reasonably provide enablement for any non-mouse transgenic animals comprising a nestin regulatory sequence" and that it "does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims" (Office Action, pages 3-4). The Examiner further states that "production of transgenic animals with desired characteristics is highly unpredictable" (Office Action, page 4); that "the specification does not provide sufficient guidance on how to isolate, select and screen for DNA constructs comprising all nestin regulatory sequences from all mammals which would be suitable for producing transgenic mammals with the desired phenotype and utility" (Office Action, page 5); and "that it would have required undue experimentation to prepare DNA constructs which utilize all nestin regulatory sequences and to produce transgenic animals from all mammalian species which express the constructs" (Office Action, page 5).

Applicants respectfully disagree. Applicants first note that

> As long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 U.S.C. 112 is satisfied. MPEP § 2164.01(b);

and that

> The enablement requirement is met if the description enables any mode of making and using the claimed invention. Engel Industries, Inc. v. Lockformer Co., 946 F,2d 1526, 20 U.S.P.Q.2d 1300 (Fed. Cir. 1991).

In the specification as filed, Applicants provide a working example which describes in detail how to make a transgenic mouse which has integrated into its genome DNA comprising a regulatory sequence of a mammalian nestin gene operably linked to a gene coding for a fluorescent protein wherein the gene coding for the fluorescent protein is expressed in multipotent stem and progenitor cells of the transgenic mouse, progeny or embryo thereof. Applicants also provide methods for assessing whether the transgenic mammal expresses the fluorescent protein in multipotent stem and progenitor cells.

Contrary to the position taken by the Examiner (Office Action, page 4), Applicants' claimed invention does not encompass all combinations of nestin regulatory sequences and fluorescent proteins. Rather, Applicants' claimed invention encompasses only constructs which comprise a regulatory sequence of a mammalian nestin gene, which, when operably linked to a gene coding for a fluorescent protein, directs expression of the fluorescent protein in multipotent stem and progenitor cells of the non-human transgenic mammal, progeny or embryo thereof.

It is respectfully submitted that the specification, drawings and working examples provide ample guidance regarding mammalian nestin genes and their regulatory sequences. Furthermore, Applicants note that nucleotide sequences of rat and human nestin genes are disclosed in U.S. Patent No. 5,338,839, (Reference AA). High sequence similarities in the rat and human nestin second introns are discussed, for example, by Lothian and Lendahl, "An Evolutionarily Conserved Region in the Second Intron of the Human Nestin Gene Directs Gene Expression to CNS Progenitor Cells and to Early Neural Crest Cell," *Eur. J. Neurosci., Vol: 9:* 452-462 (1997), (Reference AV). Mouse nestin cDNA is discussed by Kachinsky, *et al.*, Intermediate Filaments in Cardiac Myogenesis: Nestin in the Developing Mouse Heart, *J. Histochem. Cytochem., Vol 43(8):* 843-847 (1995), (Reference AV2).

Genes coding for fluorescent proteins also are extensively taught in the specification. Moreover, genes encoding fluorescent proteins are described in U.S. Patents Nos. 5,491,084 (Reference AC) and 5,804,387, (Reference AD) as well as in the article by Chiocchetti, *et al.*,

Biochim. Biophys. Acta, Vol. 1352(2): 193-202, (1997), cited by the Examiner elsewhere in the Office Action.

Based upon the guidance provided in the specification as filed and the knowledge in the art of regulatory sequences of the nestin gene and genes coding for fluorescent proteins, a skilled person is clearly enabled to isolate, screen and select other DNA constructs comprising mammalian nestin regulatory sequences which would be suitable for producing a non-human transgenic mammal such as that claimed by Applicants.

Furthermore, Applicants respectfully submit that:

> The test [for undue experimentation] is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed to enable the determination of how to practice a desired embodiment of the invention claimed. Ex Parte Forman, 230 USPQ 546, 547 (CAFC 1986).

Citing a number of references, the Examiner states that "with respect to the unpredictability of transgene expression levels sufficient to confer a particular phenotype due to species differences and/or specific elements within the transgene construct, it would have required an undue amount of experimentation to extend these results obtained in mice to levels of transgene product in any and all non-human transgenic mammals expressing transgenes encoding any and all fluorescent proteins, the consequences of that production, and therefore, the resulting phenotype" (Office Action, page 6).

The Examiner states that Ebert, *et al.* (Molecular Endocrinology, 1988) "disclose the production of transgenic mice expressing human somatotropin regulated by the mouse metallothionein promoter at levels sufficient to cause an increase in growth ; however, expression of the same transgene in pigs did not produce pigs exhibiting the same phonotypic result" (Office Action at pages 5-6). Applicants respectfully note that their claimed invention is not directed to phenotypical traits, which, as known in the art, become apparent much later in the trangenic animal's development and reflect how it physiologically responds to the transgene. Rather, Applicants' claimed invention is directed to gene expression of a fluorescent protein in particular cells of a transgenic mammal, not a particular phenotype conferred by the expressed protein.

Applicants respectfully submit that, at the time the application was filed, it was well

within the state of the art to produce non-human transgenic mammals other than mice (e.g., pig, sheep, cattle). This is clearly indicated in references cited by the Examiner.

Wall (Theriogenology, 1996) repeatedly states that, although expensive and suffering from inefficiencies, the technology for making transgenic livestock is within the state of the art. At page 58, for instance, the reference states that "[t]he limited publication record for transgenic livestock species reflects the high cost and technical difficulties associated with producing transgenic livestock more than lack of applicability". At page 64, Wall states that "[t]he tools for gene transfer are in hand, albeit the process is inefficient". At page 65 Wall states that "[t]o entice other scientists, the efficiency of producing transgenic farm animals will have to be improved. But the horizon looks bright. Many recently trained animal scientists are now equipped with the knowledge and technical skills needed to advance this technology."

Similarly, as early as 1992, Kappel, *et al.*, (Current Opinion in Biotechnology, 1992) state that "[t]ransgenic animal technology is now well established as a critical method for analyzing gene expression and function." See Kappel, *et al.* at page 551.

The Examiner directs Applicants' attention to the discussion of "position effects" in Mullins and Mullins (J. Clin. Invest.). Mullins and Mullins teach that position effects "can have major consequences on the expression of the transgene" which "is of greater concern in nonmurine transgenesis where the investment is higher" (Mullins and Mullins, p.537, 2nd column, emphasis added). However, Mullins and Mullins also clearly teach that position effects can be overcome and improved expression of heterologous genes can be achieved using a number of elements which "have been shown to function across species barriers" (Mullins and Mullins, p. 532, 2nd col.). For example, Mullins and Mullins teach that "simply including large amounts of flanking sequences may be sufficient to overcome position effects and direct expression to specific tissues" (Mullins and Mullins, p.537, 2nd column, emphasis added.)

Through their experimental approach, Ebert, *et al.*, (Molecular Endocrinology, 1988) were able to address problems cited by the Examiner at pages 5-6 in the Office Action. At page 281, the reference clearly states that the studies discussed in their article indicate that major phenotypic changes can be produced in transgenic livestock through expression of microinjected fusion genes.

Even as early as 1986, the experiments presented in the Hammer, *et al.* (J. Anim. Sci., 1986) article demonstrated "the feasibility of introducing foreign genes into the genome of several animal species by microinjection of eggs". (Hammer, *et al.*, absract).

Applicants respectfully submit that, at the time the application was filed, it would not have required undue experimentation to extend Applicants' results which were obtained in mice, to produce other non-human transgenic mammals having integrated into their genome DNA comprising a regulatory sequence of a mammalian nestin gene operably linked to a gene coding for a fluorescent protein wherein the gene coding for the fluorescent protein is expressed in multipotent stem and progenitor cells of the non-human transgenic mammal, progeny or embryo thereof.

It is respectfully submitted that Claims 1-17 and 19-24 meet the requirements of 35 U.S.C. § 112, first paragraph.

Rejection of Claims 1-17 and 19-24 Under 35 U.S.C. § 102(b)

Claims 1-17 and 19-24 are rejected under 35 U.S.C. § 102(b) as being anticipated by Zimmerman *et al.*, *Neuron Vol. 12(1):* 11-24, (1994), as evidenced by Hogan *et al.*, *Manipulating the Mouse Embryo: A Laboratory Manual*, (Cold Spring Harbor Laboratory, 1986).

Applicants' invention is directed to fluorescent proteins, as described at page 10, line 25 to page 11, line 11 of the specification. As known in the art, and as discussed also at page 16, lines 22-26, of the specification, "fluorescent proteins" have excitation and emission spectra which differ from the fluorescence spectra of tryptophan residues. Thus the fluorescence (excitation/emission spectral features) of "fluorescent proteins" are distinguishable from the fluorescence of tryptophan in a protein. However, to expedite prosecution, Applicants' present claims specifically recite that the fluorescent protein is a marker fluorescent protein.

Furthermore, Applicants respectfully submit that the differences between the *lac-Z* reporter gene and genes coding for a fluorescent protein are well established. The Examiner's attention is directed, for instance, to the article by Chiocchetti, *et al.*, Biochim. Biophys. Acta *Vol. 1352(2):* 193-202, (1997). Chiocchetti, *et al.* clearly teach that unlike fluorescent proteins, reporter genes such as *lac-Z* "rely on the addition of exogenous substrates, coenzymes or antibodies to detect reporter gene activity (Chiocchetti, *et al.* page 193, col.1). Differences

between methods using fluorescent proteins and fusion proteins with coding sequences for β galactosidase also are discussed at column 1, lines 16-24 of U.S. Patent No. 5,491,084 (Reference AC) which states that:

> [s]everal methods are available to monitor gene activity and protein distribution within cells. These include the formation of fusion proteins with coding sequences for β-galactosidase (21), and luciferases (21). The usefulness of these methods is often limited by the requirements to fix cell preparations or to add exogenous substrates or cofactors.

Accordingly, Applicants respectfully submit that Claims 1-17 and 19-24, particularly as amended, are not anticipated by Zimmerman *et al.*, as evidenced by Hogan *et al.*

Rejection of Claims 1-24 Under 35 U.S.C. § 103(a)

Claims 1-24 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Zimmerman, *et al.*, *Neuron Vol. 12(1):* 11-24, (1994), in view of Chiocchetti, *et al.*, Biochim. Biophys. Acta *Vol. 1352(2):* 193-202, (1997).

The Examiner states that Zimmerman, *et al.* "teaches transgenic mice comprising a construct containing a *lac Z* reporter transgene under the control of the promoter and second intron enhancer of the rat nestin gene" and that the reference "does not teach a construct comprising green fluorescent protein". (Office Action at page 8.) The Examiner further states that Chiocchetti, *et al.* "teaches the use of green fluorescent protein as a reporter gene, in lieu of *lac Z*, for use in transgenic animals" and that "[i]t would have been obvious to one of skill in the art at the time of the invention to substitute the green fluorescent protein coding sequence of Chiocchetti for *lac Z* in the construct of Zimmerman". (Office Action at page 8.) The Examiner states that "[o]ne would have been motivated to do so because Chiocchetti teaches that green fluorescent protein is a more powerful and sensitive tool for studying gene expression than is *lac Z*". (Office Action at page 8.)

Applicants respectfully disagree. In discussing obviousness, the court has stated that:

> "[o]bviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching[,] suggestion or incentive supporting the combination" (*In re Geiger*, 815 F.2d 686, 688, 2 U.S.P.Q.2d 1276, 1278 (Fed. Cir. 1987)) and that "[i]t is impermissible . . . simply to engage in a hindsight reconstruction of the claimed invention, using the applicant's structure as a template and selecting elements from references to fill the gaps. . . . The references themselves must provide some teaching whereby the applicant's

> combination would have been obvious." (*In re Gorman*, 18 U.S.P.Q.2d 1885, 1888 (Fed. Cir. 1991)).

That is, the issue is "whether the teachings of the prior art would, *in and of themselves and without the benefit of appellant's disclosure,* make the invention as a whole, obvious" (*In re Sponnoble* 160 USPQ 237 at 243 (CCPA 1969)). As the court also made clear in *In re Sponnoble,* "[t]he court must be ever alert not to read obviousness into an invention on the basis of the applicant's own statements; that is we must view the prior art without reading into that art applicant's teachings (*In re Sponnoble* 160 USPQ 237 at 243 (CCPA 1969)).

The problem addressed by Zimmerman, *et al.* is the identification of regulatory elements in the nestin gene that direct transgene expression in neural stem cells and muscle precursors. The reference discloses mouse embryos or a P1 newly born pup comprising a Lac Z reporter gene under the control of regulatory sequences of the rat nestin gene. X-gal expression is observed in gross features or subdivisions of the embryo or newly born pup organisms studied.

Zimmerman, *et al.* neither teach nor suggest a non-human transgenic mammal, progeny or embryo thereof which has integrated into its genome DNA comprising a regulatory sequence of a mammalian nestin gene operably linked to a gene coding for a fluorescent protein wherein the gene coding for the fluorescent protein is expressed in multipotent stem and progenitor cells of the non-human transgenic mammal, progeny or embryo thereof. The reference neither teaches nor suggests a method for producing such a non-human transgenic mammal.

Furthermore, there is no disclosure or suggestion in Zimmerman, *et al.* of a method for measuring a multipotent stem and progenitor cell population in a live and/or adult animal or an animal organ or region thereof which comprises measuring cells which fluoresce from the organ or region thereof of a non-human transgenic mammal which has integrated into its genome DNA including a regulatory sequence of a mammalian nestin gene operably linked to a gene coding for a fluorescent protein, wherein the gene coding for the fluorescent protein is expressed in multipotent stem and progenitor cells of the non-human transgenic mammal, wherein the cells which fluoresce are multipotent stem and progenitor cells.

Chiocchetti, *et al.* teach mice in which the green fluorescent protein of jellyfish *Aequorea victoria* is placed under the control of two regulatory regions: (a) the hemopexin promoter which drives high expression of hemopexin in human adult liver and a weaker expression in some brain

districts and (b) the β1-integrin distal promoter which drives ubiquitous expression during the mouse embryonic development.

Chiocchetti, *et al.* do not remedy the deficiencies of Zimmerman, *et al.* More specifically, there is no disclosure or suggestion in Chiocchetti, *et al.* of a non-human transgenic mammal, progeny or embryo thereof which has integrated into its genome DNA comprising a regulatory sequence of a mammalian nestin gene operably linked to a gene coding for a fluorescent protein wherein the gene coding for the fluorescent protein is expressed in multipotent stem and progenitor cells of the non-human transgenic mammal, progeny or embryo thereof. As with Zimmerman, *et al.* the reference neither teaches nor suggests a method for producing such a non-human transgenic mammal.

Furthermore, there is no disclosure or suggestion in Chiocchetti, *et al.* of a method for measuring a multipotent stem and progenitor cell population in a live animal or an animal organ or region thereof which comprises measuring cells which fluoresce from the organ or region thereof of a non-human transgenic mammal which has integrated into its genome DNA including a regulatory sequence of a mammalian nestin gene operably linked to a gene coding for a fluorescent protein, wherein the gene coding for the fluorescent protein is expressed in multipotent stem and progenitor cells of the non-human transgenic mammal, wherein the cells which fluoresce are multipotent stem and progenitor cells.

As discussed throughout the specification and in particular at page 5, lines 14 through page 6, line 3, Applicants' invention addresses problems and objectives not faced by the authors of the cited references.

As noted above, the teaching of Zimmerman, *et al.* is not directed to the problem of studying or measuring multipotent stem and progenitor cells, nor in producing a non-human transgenic mammal that would allow studying multipotent stem and progenitor cells and indeed measuring their presence. Therefore, it is respectfully submitted that there is no motivation in Zimmerman, *et al.* for replacing the *lac Z* reporter gene with a gene coding for fluorescent protein.

The problem addressed by Chiocchetti, *et al.* also is different from that faced by Applicants. Chiocchetti, *et al.* does not concern itself with nestin expression, stem and progenitor cells, their presence in an animal or organ thereof, or with producing a transgenic

mammal that would allow studying multipotent stem and progenitor cells and indeed measuring their presence. Contrary to the position taken by the Examiner, Applicants respectfully submit that there is no motivation, explicit or implied, in Chiocchetti, *et al.* to operably link a regulatory sequence of a mammalian nestin gene to a gene coding for a fluorescent protein wherein the gene coding for the fluorescent protein is expressed in multipotent stem and progenitor cells of a non-human transgenic mammal, progeny or embryo thereof.

Accordingly, it is respectfully submitted that Claims 1-24 meet the requirements of 35 U.S.C. § 103(a).

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (781) 861-6240.

Respectfully submitted,

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Dated: April 4, 2001

MARKED UP VERSION OF AMENDMENTS

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

1. (Amended) A non-human transgenic mammal, progeny or embryo thereof which has integrated into its genome DNA comprising a regulatory sequence of a mammalian nestin gene operably linked to a gene coding for a marker fluorescent protein wherein the gene coding for the marker fluorescent protein is expressed in multipotent stem and progenitor cells of the non-human transgenic mammal, progeny or embryo thereof.

2. (Amended) The non-human transgenic mammal, progeny or embryo thereof of Claim 1 wherein the gene coding for the marker fluorescent protein is selectively expressed in multipotent stem and progenitor cells of the non-human transgenic mammal or progeny thereof.

3. (Amended) The non-human transgenic mammal, progeny or embryo thereof of Claim 1 wherein the gene coding for the marker fluorescent protein is expressed in neural stem and progenitor cells of the non-human transgenic mammal or progeny thereof.

9. (Amended) A method of producing a non-human transgenic mammal which expresses a marker fluorescent protein in multipotent stem and progenitor cells, comprising:

(a) introducing into a fertilized egg of a non-human mammal, DNA comprising a regulatory sequence of a mammalian nestin gene operably linked to a gene coding for a marker fluorescent protein that is expressed in multipotent stem and progenitor cells of the non-human mammal;

(b) introducing the fertilized egg of (a) into a non-human mammal of the same species;

(c) allowing the non-human mammal to produce progeny which are non-human transgenic mammals; and

(d) selecting non-human mammal progeny of (c) whose multipotent stem and progenitor cells express the marker fluorescent gene.

10. (Amended) The method of Claim 9 wherein the gene coding for a marker fluorescent protein is selectively expressed in multipotent stem and progenitor cells.

11. (Amended) The method of Claim 9 wherein the gene coding for a marker fluorescent protein is expressed in neural stem and progenitor cells.

19. (Amended) A method for measuring a multipotent stem and progenitor cell population in an animal organ or region thereof, comprising:
measuring cells which fluoresce from the organ or region thereof of a non-human transgenic mammal which has integrated into its genome DNA comprising:
a regulatory sequence of a mammalian nestin gene operably linked to a gene coding for a fluorescent protein, wherein the gene coding for the fluorescent protein is expressed in multipotent stem and progenitor cells of the non-human transgenic mammal,
wherein the cells which fluoresce are multipotent stem and progenitor cells.

22. (Amended) The [non-human transgenic mammal, progeny or embryo thereof] method of Claim 19 wherein the regulatory sequence includes a second intron sequence of the mammalian nestin gene.

23. (Amended) The [non-human transgenic mammal, progeny or embryo thereof] method of Claim 19 wherein the regulatory sequence further includes a promoter.

24. (Amended) The [non-human transgenic mammal, progeny or embryo thereof] method of Claim 23 wherein both the promoter and the regulatory sequence are obtained from the same mammalian nestin gene.